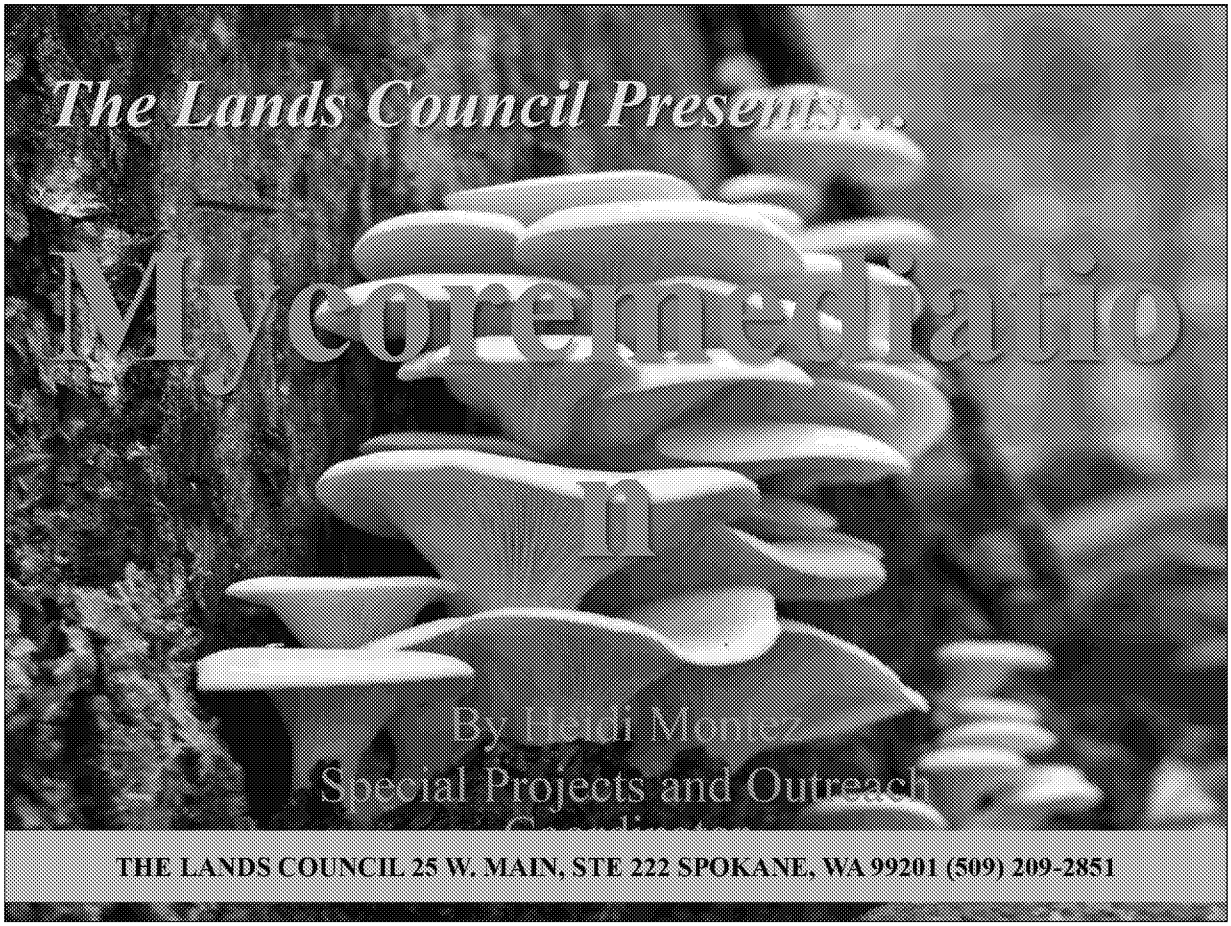

The Lands Council

Environmental Non-Profit
Spokane, WA

We preserve and revitalize Inland Northwest forests, water, and wildlife through advocacy, education, effective action, and community engagement.

We collaborate with a broad range of interested parties to seek smart and mutually respectful solutions to environment and

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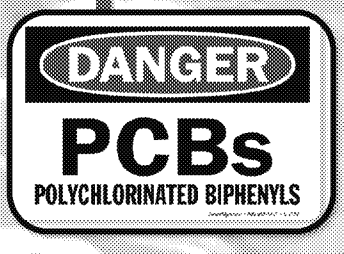
Project Development

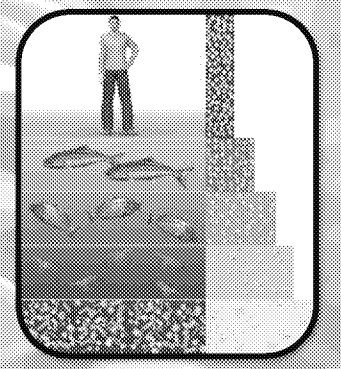
PCBs & the Spokane River:

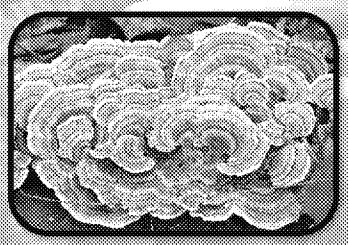
- Challenging issue
- 209 congeners (legacy/ inadvertant)
- Many sources and pathways
- Persistent
- Bioaccumulate
- Toxic



Current/ Industrial clean up methods:

- expensive
- huge environmental impacts
- only moving the PCBs, not destroying them





Is there a natural solution?

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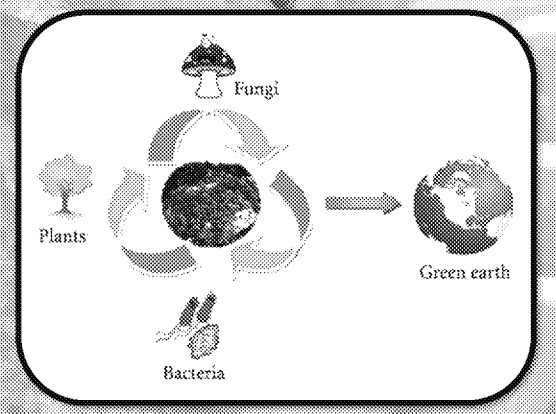
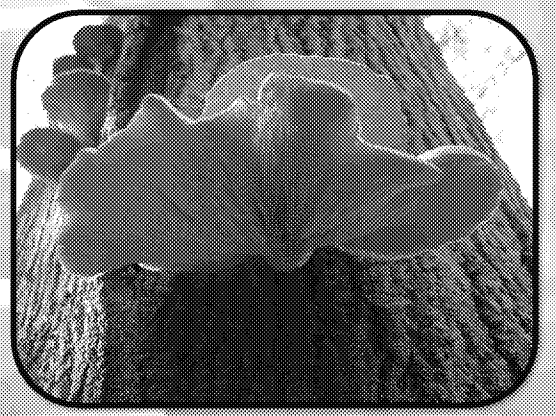
What is Mycoremediation?





"Bioremediation"
using naturally occurring organisms
to detoxify the environment

"Mycoremediation"
using fungi (mushrooms) to
detoxify the environment

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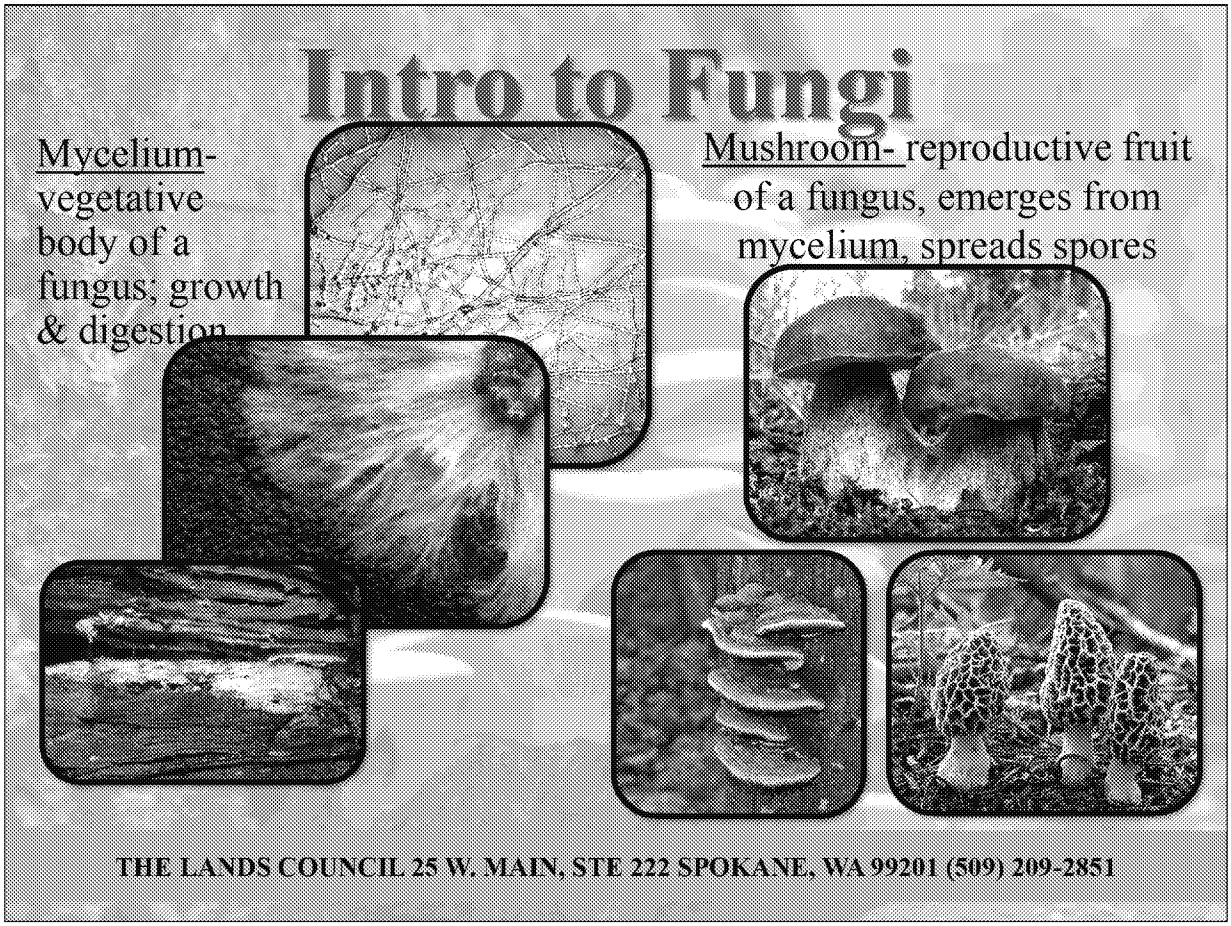
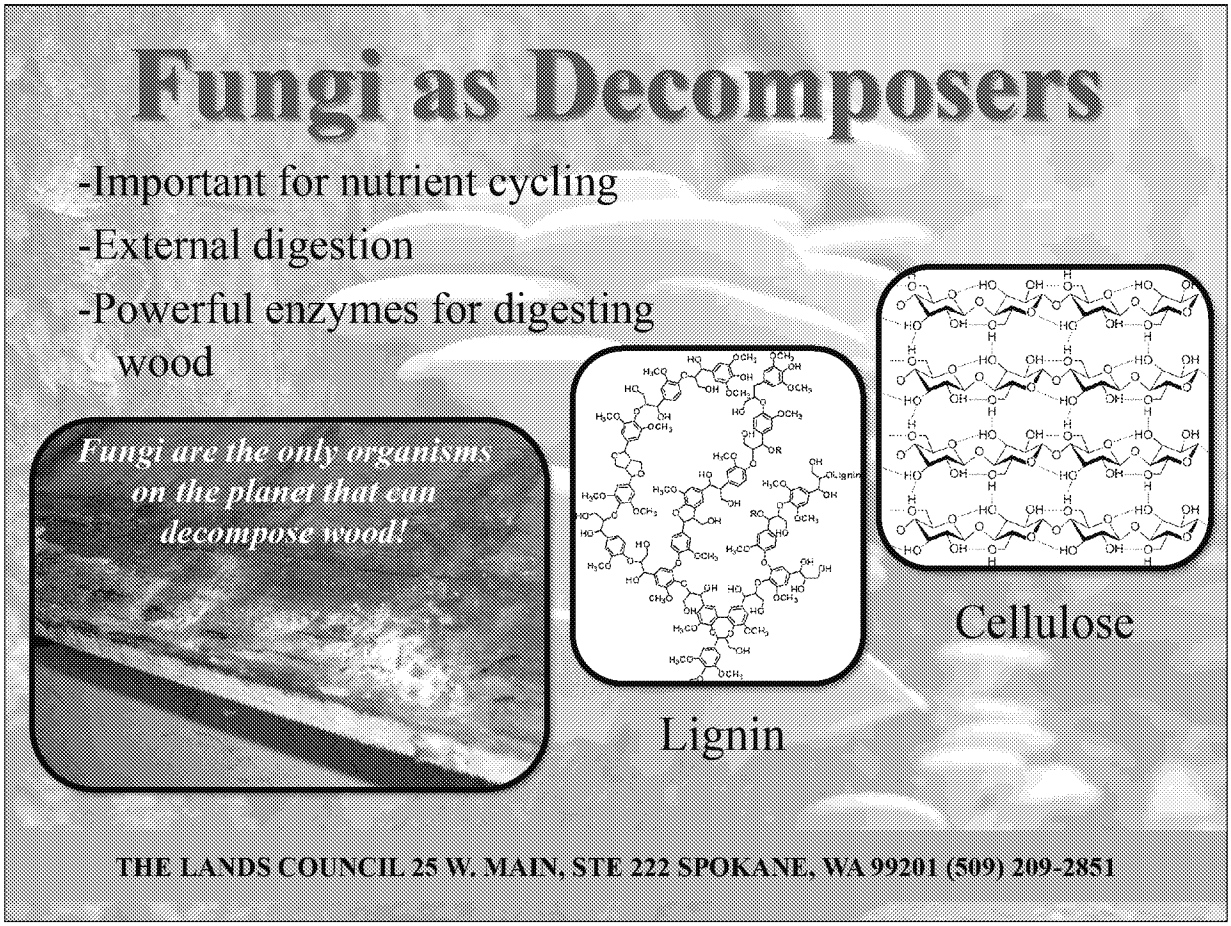
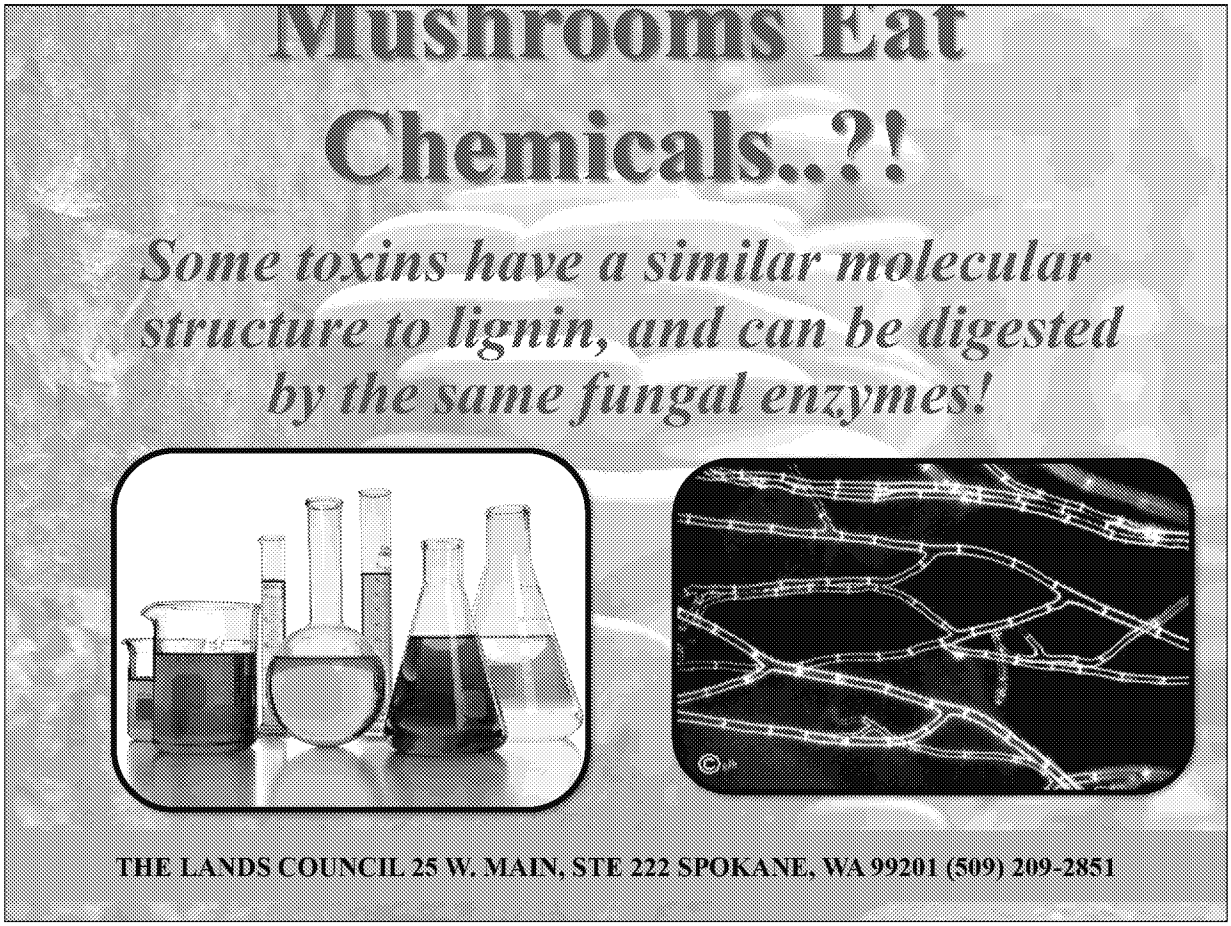


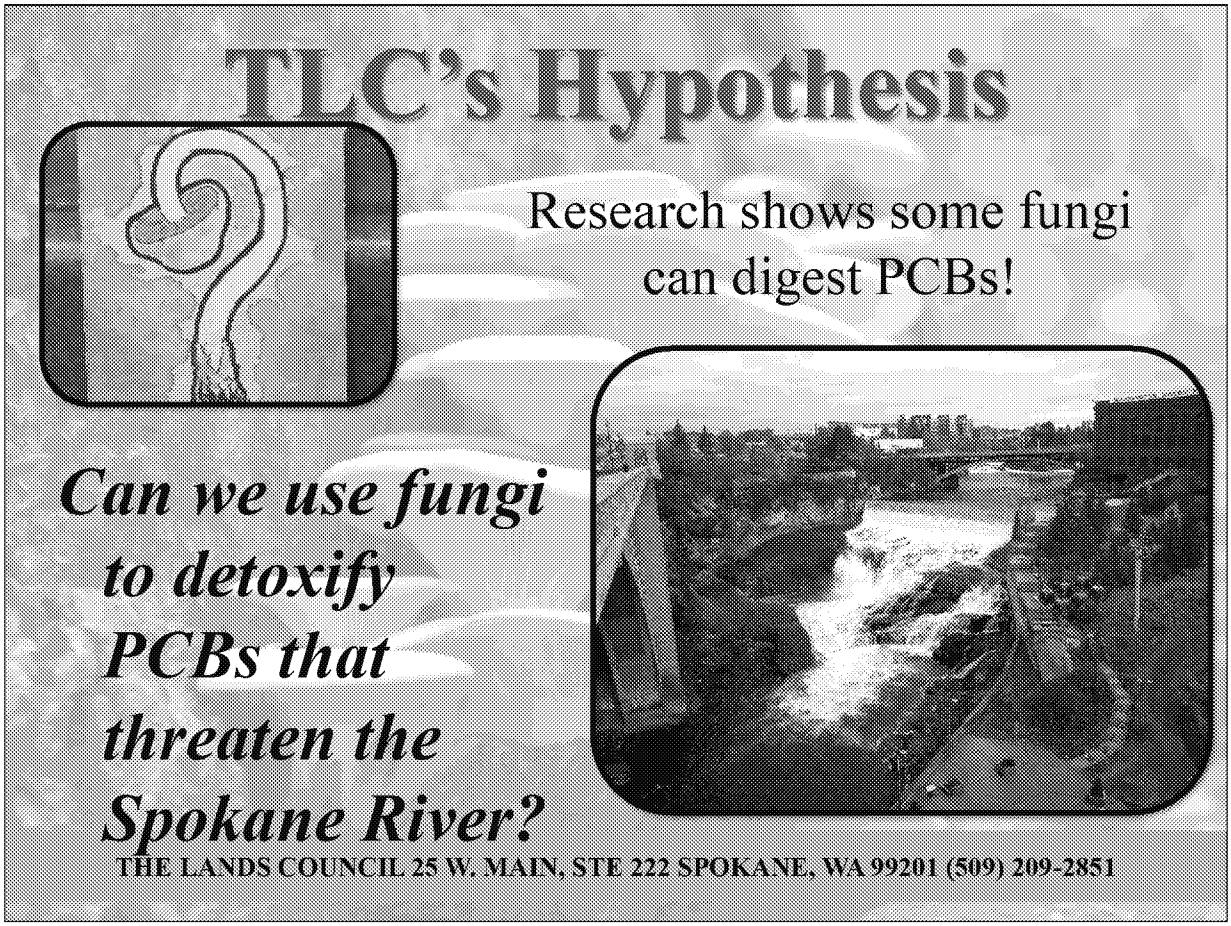
Before we get into the project, we need to understand how fungi grow.
Fungi need to eat food to get energy & breathe oxygen, similar to how animals do. Mycelium is the white webby stuff you might find under a log in the forest. This is the vegetative body of the fungus that carries out growth, metabolism, immune responses etc. Mycelium is really the whole organism, and the mushroom is the reproductive fruit whose purpose is to spread spores. Think of an apple tree; the mycelium is the roots, trunk, branches and leaves, and the mushroom is the apple.



Decomposing fungi are very important for nutrient cycling in the ecosystem. Fungi need to eat to get their energy, but they eat very differently than animals. They secrete enzymes outside of themselves, the enzymes do their work on the food, and then they absorb the nutrients they want and need. They have very powerful enzymes that can digest tough materials. Fungi are actually the only organisms on earth that can break down wood. They have very specialized enzymes for breaking down the lignin and cellulose in wood.



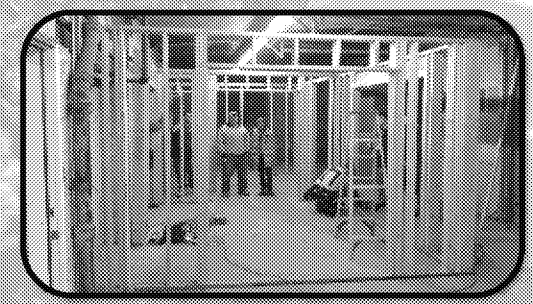
It turns out that some toxic chemicals have a very similar molecular structure to lignin, and can actually be broken down by lignin degrading enzymes!

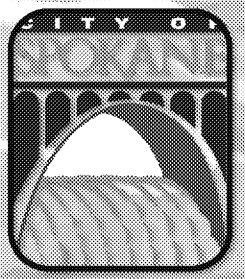


One of these tough chemicals is PCBs, and research shows that some wood-rotting fungi can successfully break down PCBs as well. At the Lands Council, we are investigating whether the natural digestive abilities of these fungi can help us clean up the Spokane River.

Mycology Program

- TLCs newest program
- Partnered with Fulcrum Institute and received a Community Development Block Grant to build new Lab
  - ESD101 construction
- Partnered with the City of Spokane to conduct the first Mycoremediation experiment in Spokane
- Formed a Technical Advisory Committee
  - Experts in diverse fields overseeing project





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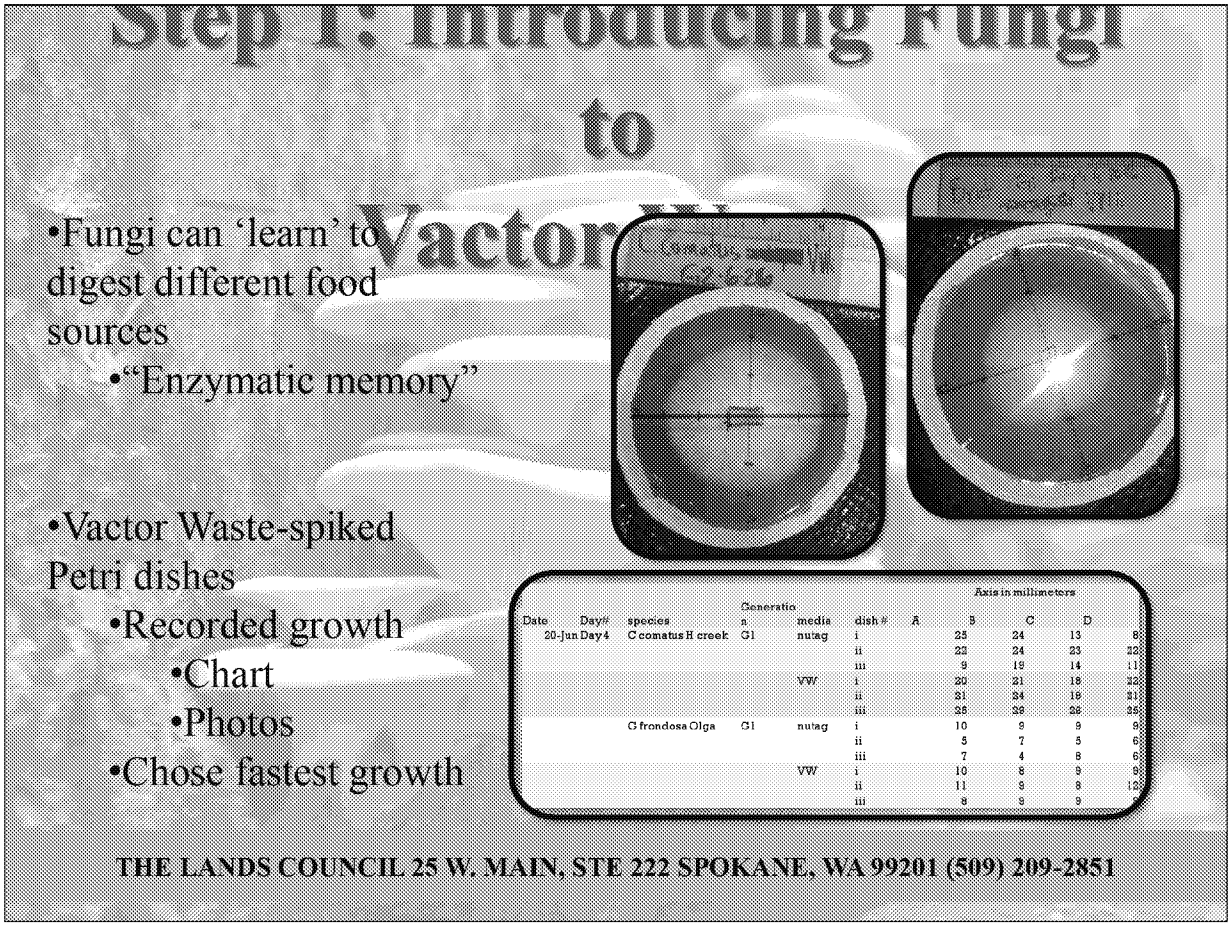
We partnered with the City of Spokane to do a really exciting experiment that could help clean up the Spokane River. Vactor Waste, aka storm drain sludge, contains lots of pollutants, including PCBs. We are growing 8 species of fungi and will 'feed' them Vactor Waste, to see if they can eat and break down the PCBs. Mycoremediation is a groundbreaking field in biology, ant there have been very few real world studies, do it is very exciting to be working on this.

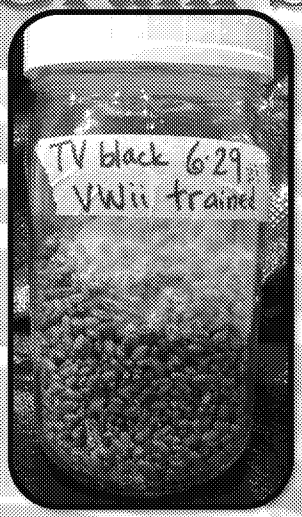
Coprinus comatus
Grifola frondosa
Lentinula edodes
Pleurotus ostreatus brat
Pleurotus ostreatus columbinus
Pleurotus wild CA creek
Stropharia rugosoannulata
Trametes versicolor

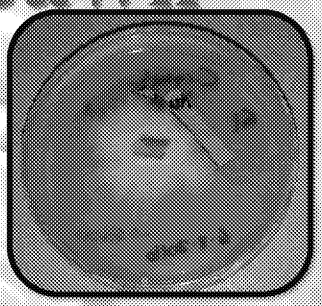


There's evidence that fungi can actually learn to digest different food sources if they are introduced to them at an early growth stage. When the mycelium encounters a food source, it will pull the appropriate enzymes out of its DNA so it can eat that food. As it continues to grow, the fungus will remember encountering that food, in case it needs to eat it again. So, I spiked some petri dishes with a very small amount of vactor waste, to let the mycelium get a taste for it. I grew each species on 3 of these dishes, watched them grow, and recorded their growth in a chart. I chose the fastest growing culture from each species to use for the next steps.

Step 2: Grain Spawn

- Each species: 2 jars of hydrated/ sterilized grain
- Incubated at 75 degrees F for one month
- Recorded growth
  - Chart
  - Photos
- Chose fastest growth





Date: July 13, 2016 Day 14 Jar #1

Species	% growth on grain surface: Upper	vertical	base	density (i, ii, iii)
Cyanus comelus hunter crest	60	10	0	ii
Grifola frondosa	66	20	0	ii
Lentinula edodes	70	30	0	ii
Pleurotus ostreatus brut	100	50	0	ii
Pleurotus ostreatus columbinus	100	30	0	ii

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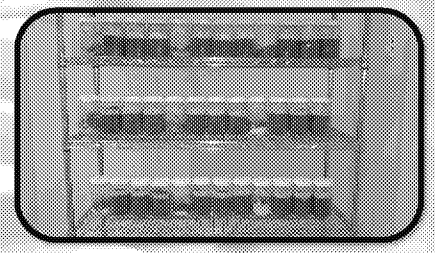
Step 2 was to take cuttings from the VW spiked petri dish, and transfer them to 2 jars of hydrated, sterilized rye grain. These fungi love to grow on the nutritious whole grains. I incubated them at 75 Degrees for a month, measuring and recording their growth. After a month, I used the growth charts to select the faster of each species, to transfer to the next step.

Step 3: Sawdust Spawn

- Transferred to 3 jars of hydrated/ sterilized alder sawdust
- Currently incubating at 75 degrees F
- Measuring/ recording growth



Species	Jar #1		
Date: August 2, 2010	% growth	density	notes
Cyptotrama asprata hunter creek	20		myc just by grains
Gyrfola frondosa	50		
Lentinula edodes	75		
Pleurotus ostreatus brad	100	i-ii	
Pleurotus ostreatus columbianus	50		
Pleurotus wild CA creek	100	i-ii	
Stropharia rugosoannulata	100	i-ii	
Tremella reticulata black	100	ii-iii	mostly iii

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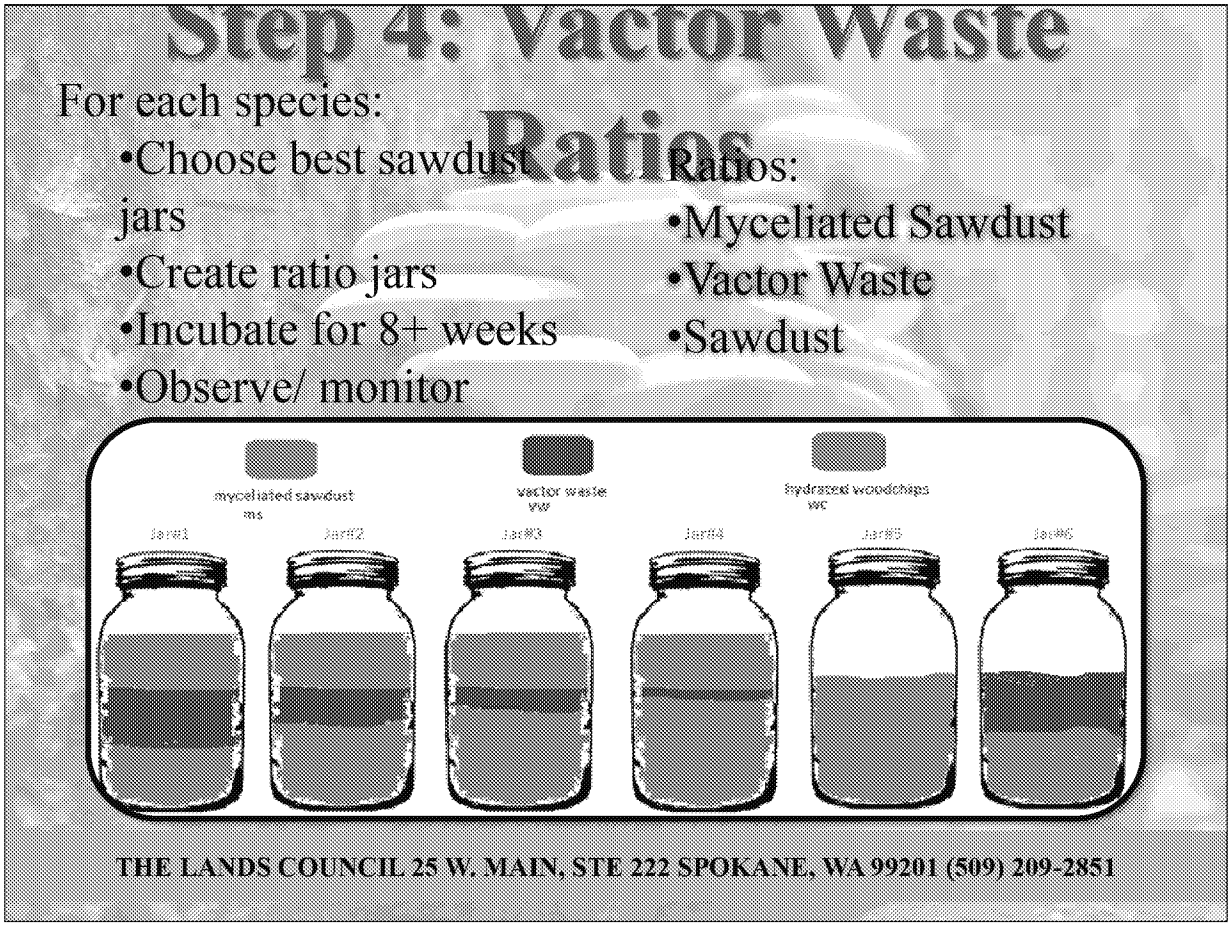
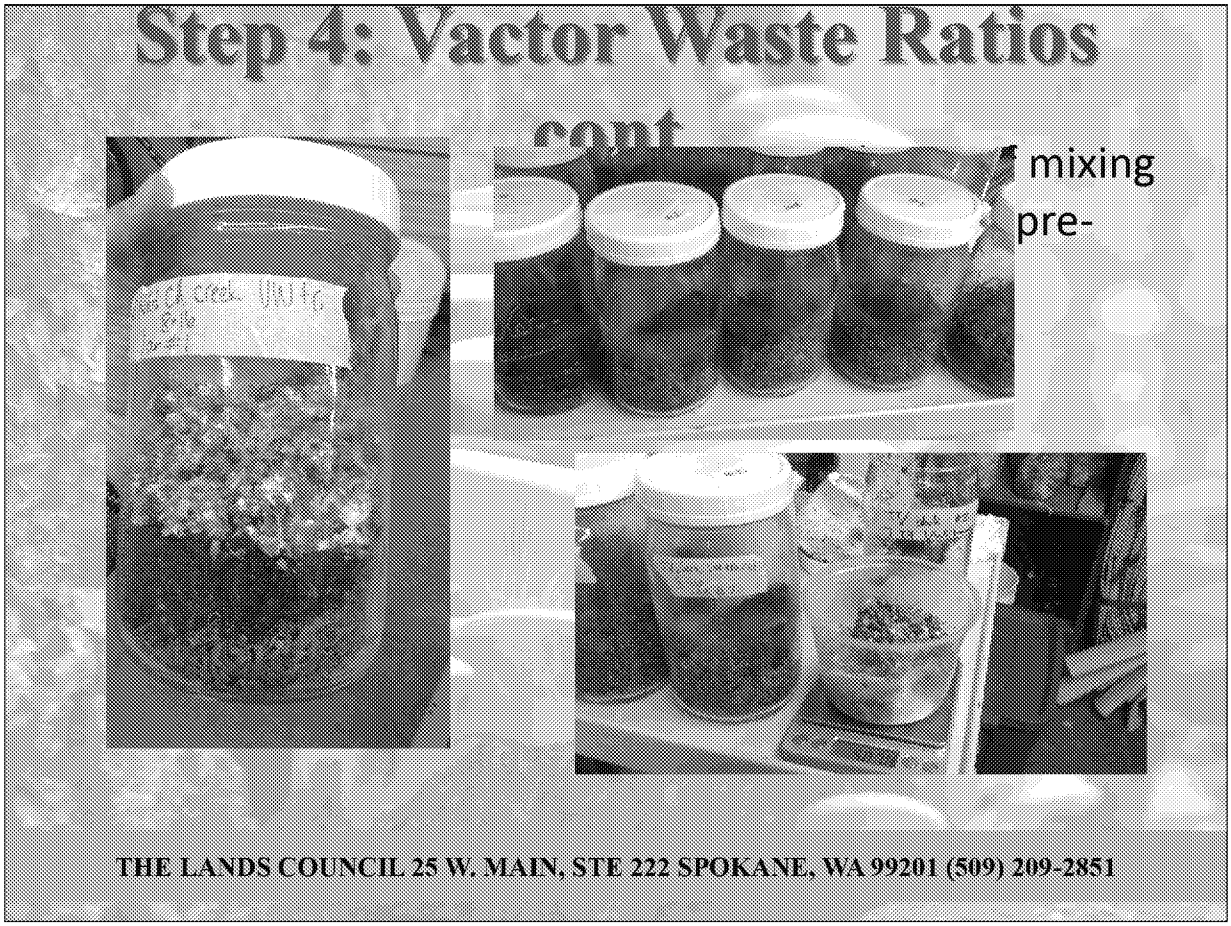
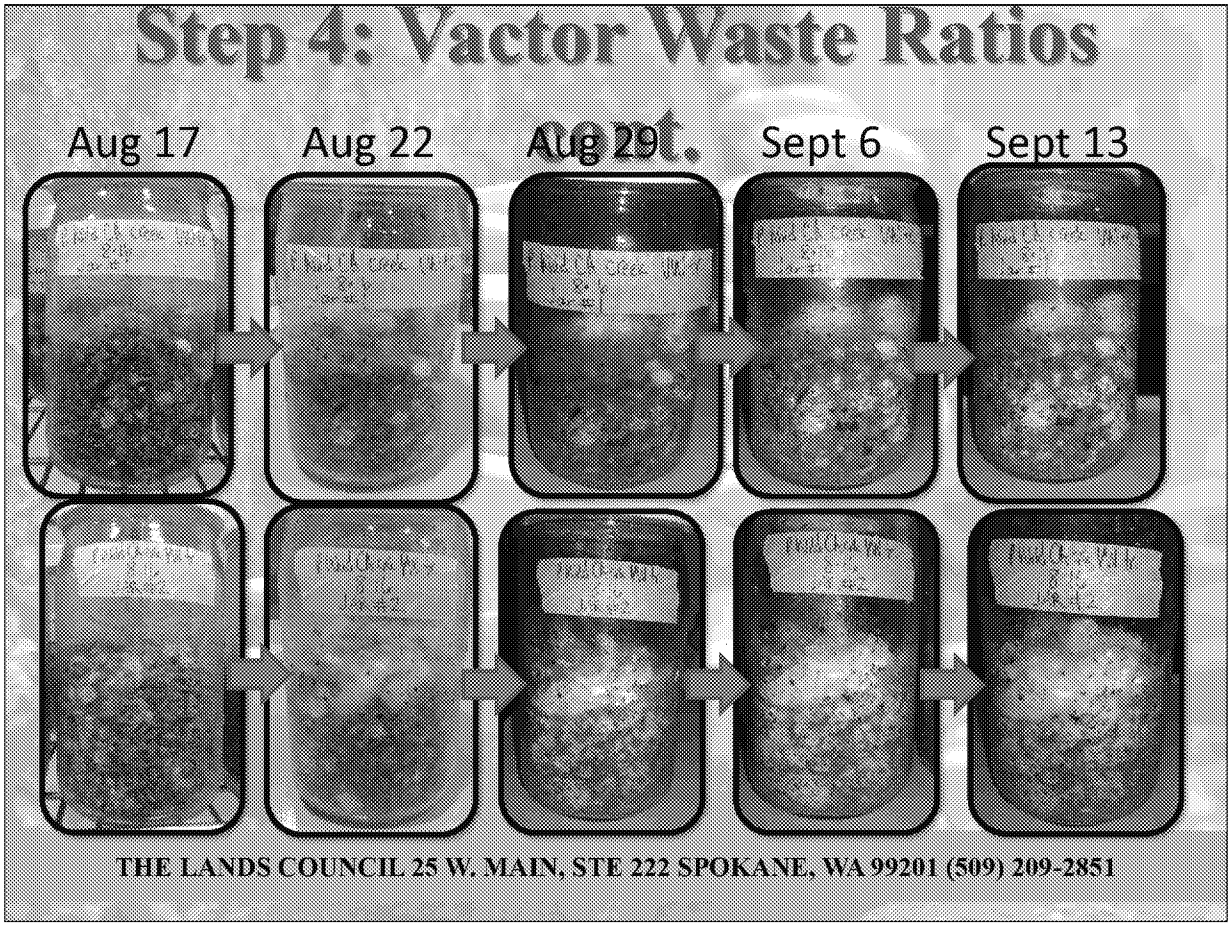
This is where I am right now in the experiment. About two weeks ago, I took the fastest growing grain jar of each species, and transferred it to three jars of hydrated/ sterilized alder sawdust. The sawdust spawn is currently incubating at the lab, and I'm recording their growth in a chart. This week, Ill start preparing the substrate for the next step.



This is where the experiment gets really exciting. For each species, I will choose the fastest growing healthiest sawdust spawn, and mix it with these ratios of Vactor waste and more sawdust. The reasoning behind the various ratios is that we aren't sure what the fungi are going to do with the Vactor Waste, what amount might be too toxic for them, etc. This will be a great way to visually observe what the fungi are doing, and we will be able to learn a great deal just from that visual observation. The last two jars are the controls: a biotic control, and an abiotic control.



The fungi are currently incubating in the jars of Vactor waste and sawdust in the lab. We are about half way through this stage, letting the fungi grow and digest as much as they can inside their jars.



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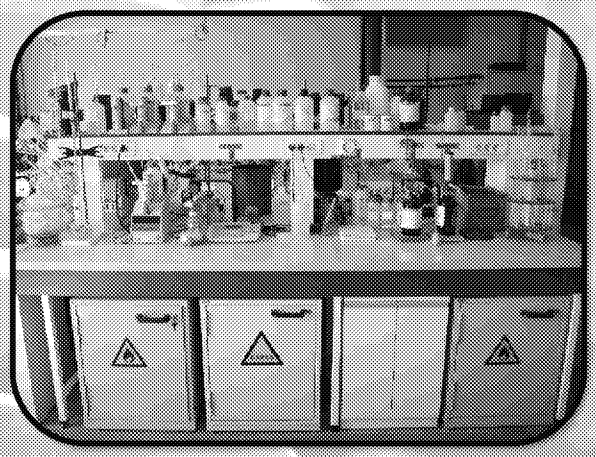
Step 3: Analytical Testing

- Testing before fungal treatment
  - Vactor Waste/ substrate base characterization
  - Still waiting for results
- Testing after fungal treatment
  - Select jars with best growth
  - Send samples for PCB testing
  - Results & Final report



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The Vactor waste, sawdust and grain are all being tested for PCBs. We are still waiting on those results to come back. After the ratios jars have incubated and been given a chance to fully colonize, we will select jars that have vigorous, healthy growth. We will send samples for PCB analysis, and assess whether any of the fungi were able to reduce the levels of PCBs.

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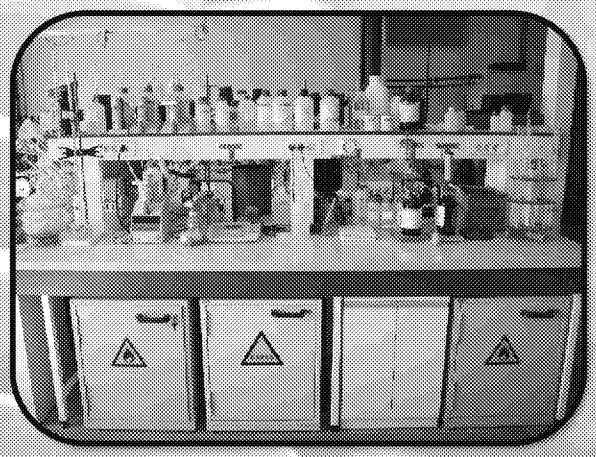

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Mycoremediation Challenges

- Groundbreaking field of biology
- Few similar projects to model after
- Living organisms- respond to environment
- PCB acquisition/ regulations
- Cost of testing

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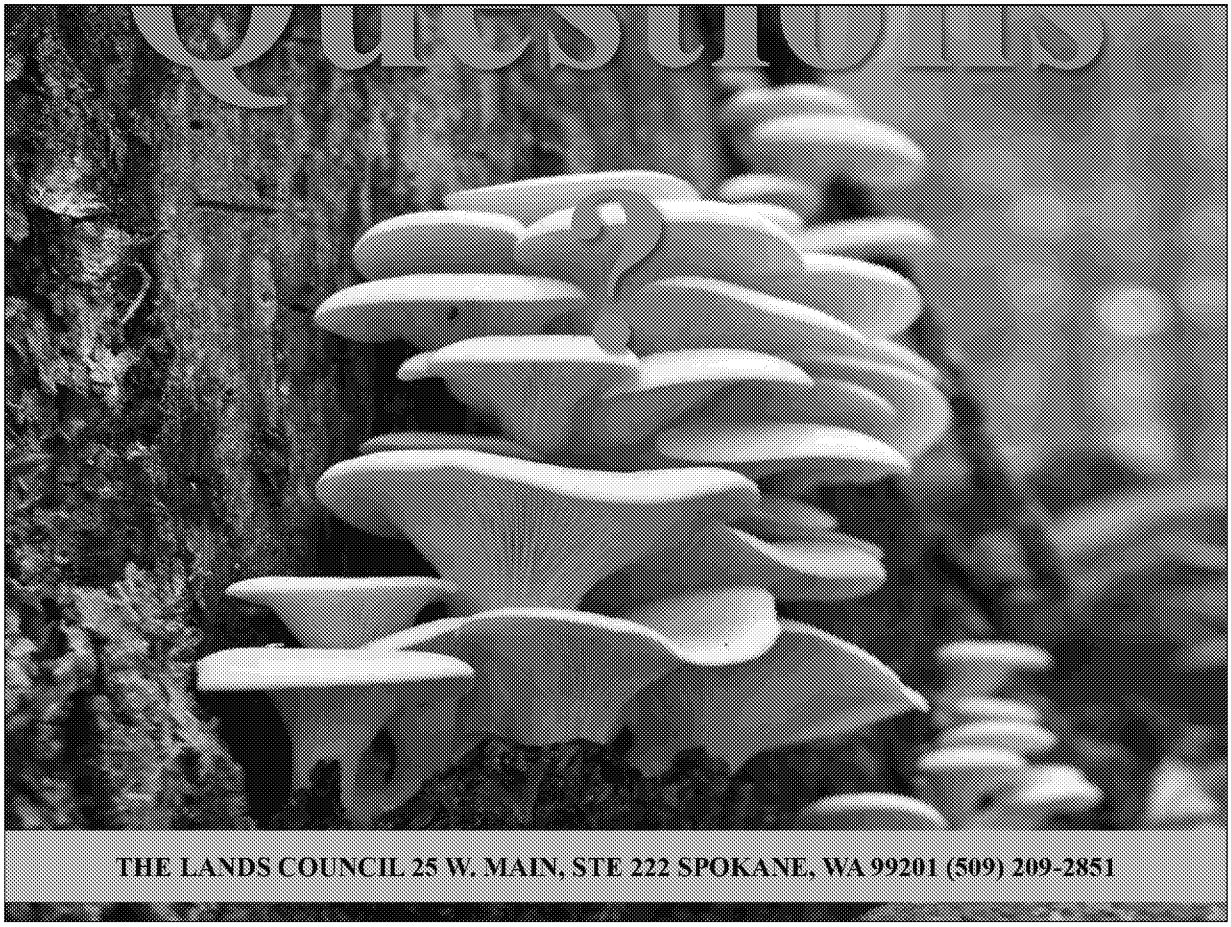
Mycoremediation is a relatively new field, so there are significant challenges. A lot of work has to be done to implement this technology, there are very few projects to model after. Although a good deal of research in highly controlled lab settings has been done, not a lot of real world experiments have been done. This is due to the high cost of scientific experiments and testing, such as PCB testing. Its very prohibitive for many researchers. Also, any type of bioremediaiton is working with living organisms, which have specific needs and conditions that have to be met; food, water, temp, air etc.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